

### **IN THE CLAIMS**

Please amend the claims as follows.

1. (Currently Amended) A method to detect the presence or amount of a first molecule for a first enzyme-mediated reaction and a second molecule for a second enzyme-mediated reaction, comprising:
  - a) contacting a sample, ~~[[with]]~~ a reaction mixture for a ~~[[the]]~~ first enzyme-mediated reaction to detect a first molecule, and a reaction mixture for ~~[[the]]~~ a second enzyme-mediated reaction to detect a second molecule, wherein a reaction mediated by a first enzyme in the first reaction yields a bioluminogenic product, and wherein a reaction mediated by a second enzyme in the second reaction yields a fluorogenic product; and
  - b) detecting the presence or amount of the first and the second molecules in the sample.
2. (Original) The method of claim 1 wherein the first molecule is a substrate for the first enzyme-mediated reaction.
3. (Original) The method of claim 1 wherein the second molecule is a substrate for the second enzyme-mediated reaction.
4. (Original) The method of claim 1 wherein the first molecule is an enzyme for the first enzyme-mediated reaction.
5. (Original) The method of claim 1 wherein the second molecule is an enzyme for the second enzyme-mediated reaction.
6. (Original) The method of claim 1 wherein the first molecule is a co-factor for the first enzyme-mediated reaction.

Title: LUMINOGENIC AND NONLUMINOGENIC MULTIPLEX ASSAY

---

7. (Original) The method of claim 1 wherein the second molecule is a co-factor for the second enzyme-mediated reaction.
8. (Previously Presented) The method of claim 1 wherein bioluminescence is employed to detect the first molecule.
9. (Original) The method of claim 1 wherein fluorescence is employed to detect the second molecule.
10. (Original) The method of claim 1 wherein the presence or amount of the first and second molecules is detected sequentially.
11. (Original) The method of claim 1 wherein the sample is a cell lysate.
12. (Original) The method of claim 1 wherein the sample is contacted with the reaction mixture for the first reaction before the reaction mixture for the second reaction.
13. (Original) The method of claim 1 wherein the sample is contacted with the reaction mixture for the second reaction before the reaction mixture for the first reaction.
14. (Original) The method of claim 1 wherein the sample is contacted with the reaction mixture for the first reaction and the second reaction at the same time.
15. (Withdrawn) A method to detect the presence or amount of a first enzyme or cofactor for a first enzyme-mediated reaction, comprising:
  - a) contacting a sample with a first substrate for the first enzyme, a second substrate for a second enzyme, and optionally a third enzyme, wherein a reaction between the first substrate and the first enzyme or a reaction between the third enzyme and a product of a

Title: LUMINOGENIC AND NONLUMINOGENIC MULTIPLEX ASSAY

---

reaction between the first enzyme and the first substrate yields a luminogenic product, wherein the second substrate and/or a product of a reaction between the second substrate and the second enzyme is/are not luminogenic; and

b) detecting the presence or amount of the first enzyme or cofactor for the reaction mediated by the first enzyme.

16. (Withdrawn) A method of assaying an enzyme-mediated luminescence reaction to detect the presence or amount of an enzyme or cofactor for a first enzyme-mediated reaction, comprising:

a) contacting a sample with a first substrate for a first enzyme, a second substrate for a second enzyme, and optionally a third enzyme, wherein a reaction between the first substrate and the first enzyme or a reaction between the third enzyme and a product of the reaction between the first enzyme and the first substrate yields a luminogenic product, wherein the second substrate and/or a product of a reaction between the second substrate and the second enzyme is/are not luminogenic; and

b) detecting luminescence.

17. (Withdrawn) The method of claim 15 wherein luminescence is detected.

18. (Withdrawn) The method of claim 15 or 16 wherein luminescence increases in the presence of the first enzyme or cofactor.

19. (Withdrawn) The method of claim 15 or 16 wherein luminescence decreases in the presence of the first enzyme or cofactor.

20. (Withdrawn) The method of claim 15 or 16 further comprising detecting the presence or amount of the second enzyme.

21. (Withdrawn) The method of claim 20 wherein fluorescence is employed to detect the presence or amount of the second enzyme.
22. (Withdrawn) The method of claim 20 wherein the presence or amount of the first enzyme or cofactor and the presence or amount of the second enzyme are detected sequentially.
23. (Withdrawn) The method of claim 20 wherein the presence or amount of the first enzyme or cofactor and the presence or amount of the second enzyme are detected simultaneously.
24. (Withdrawn) The method of claim 20 wherein the presence or amount of the second enzyme is detected colorimetrically.
25. (Withdrawn) The method of claim 20 wherein the presence or amount of the second enzyme is detected by contacting the sample with a fourth enzyme and third substrate for a reaction between the product of the reaction between the second substrate and second enzyme which yields a fluorogenic product.
26. (Withdrawn) The method of claim 15 or 16 further comprising detecting the presence or amount of the nonluminogenic second substrate or the nonluminogenic product of the reaction between the second substrate and the second enzyme.
27. (Withdrawn) The method of claim 15 or 16 wherein the second enzyme does not react substantially with the first substrate.
28. (Withdrawn) The method of claim 15 or 16 wherein the first enzyme does not react substantially with the second substrate.

29. (Withdrawn) The method of claim 15 or 16 wherein the second substrate or the product of the reaction between the second substrate and the second enzyme is fluorescent.
30. (Withdrawn – Previously Presented) The method of claim 29 wherein the second substrate or the product of the reaction between the second substrate and the second enzyme comprises ethidium bromide, fluorescein, Cy3, BODIPY™, a rhodol, Rox, 5-carboxyfluorescein, 6-carboxyfluorescein, an anthracene, 2-amino-4-methoxynaphthalene, a phenalenone, an acridone, fluorinated xanthene derivatives,  $\alpha$ -naphthol,  $\beta$ -naphthol, 1-hydroxypyrene, coumarin, 7-amino-4-methylcoumarin (AMC), 7-amino-4-trifluoromethylcoumarin (AFC), TEXAS RED™, tetramethylrhodamine, carboxyrhodamine, rhodamine, cresyl, rhodamine-110 or resorufin.
31. (Withdrawn) The method of claim 15 or 16 wherein one enzyme is a glycosidase, phosphatase, kinase, dehydrogenase, peroxidase, sulfatase, peptidase, or hydrolase.
32. (Withdrawn) The method of claim 15 or 16 wherein one enzyme is a protease.
33. (Withdrawn) The method of claim 32 wherein one enzyme is a caspase.
34. (Withdrawn) The method of claim 33 wherein the caspase includes caspase-3, caspase-7 or caspase-8.
35. (Withdrawn-Currently amended) The method of claim 15 or 16 wherein one of the substrates comprises DEVD (SEQ ID NO:9), WEHD (SEQ ID NO:10), LEHD (SEQ ID NO:11), VEID (SEQ ID NO:12), VEVD (SEQ ID NO:13), VEHD (SEQ ID NO:14), IETD (SEQ ID NO:15), AEVD (SEQ ID NO:16), LEXD (SEQ ID NO:17), VEXD (SEQ

ID NO:18), IEHD (SEQ ID NO:19), PEHD (SEQ ID NO:20), ZEVD (SEQ ID NO:21) or LETD (SEQ ID NO:22).

36. (Withdrawn-Currently amended) The method of claim 15 or 16 wherein one of the substrates comprises  $X_1$ - $X_2$ - $X_3$ -D (SEQ ID NO:23) wherein  $X_1$  is Z, Y, D, L, V, I, A, W or P,  $X_2$  is V or E, and  $X_3$  is any amino acid.
37. (Withdrawn) The method of claim 15 or 16 wherein one of the substrates is a substrate for trypsin or tryptase.
38. (Withdrawn) The method of claim 15 or 16 wherein one enzyme cleaves a substrate comprising arginine or lysine.
39. (Withdrawn) The method of claim 15 or 16 wherein the sample is a cell lysate.
40. (Withdrawn) The method of claim 39 wherein the sample is a cellular sample that is treated with a cell death inducing agent prior to lysis.
41. (Withdrawn) The method of claim 15 or 16 wherein the sample comprises intact cells.
42. (Withdrawn) The method of claim 15 or 16 wherein the third enzyme is a luciferase.
43. (Withdrawn) The method of claim 42 wherein the luciferase is a beetle luciferase.
44. (Withdrawn) The method of claim 15 or 16 wherein the second substrate is a substrate for lactate dehydrogenase.

Title: LUMINOGENIC AND NONLUMINOGENIC MULTIPLEX ASSAY

---

45. (Withdrawn) The method of claim 15 or 16 wherein the sample is contacted with the first substrate before the second substrate.
46. (Withdrawn) The method of claim 15 or 16 wherein the sample is simultaneously contacted with the first and the second substrates.
47. (Withdrawn) The method of claim 15 or 16 wherein the sample is contacted with the second substrate before the first substrate.
48. (Withdrawn) The method of claim 15 or 16 wherein the presence or amount of the cofactor is detected by contacting the sample with the first enzyme.
49. (Withdrawn) A method to detect the presence or amount of at least two molecules in a sample, comprising:
  - a) contacting the sample with a first substrate for a first enzyme, a second substrate for a second enzyme and optionally a third enzyme, wherein a reaction between the first substrate and the first enzyme or the third enzyme and a product of a reaction between the first enzyme and first substrate yields a luminogenic product, wherein the second substrate and/or a product of a reaction between the second substrate and the second enzyme is/are not luminogenic, and wherein the first and second enzymes are not the same; and
  - b) detecting the presence or amount of the first and second enzymes or a cofactor for a reaction mediated by the first or second enzyme.
50. (Withdrawn) The method of claim 49 wherein luminescence is detected.
51. (Withdrawn) The method of claim 49 wherein at least one enzyme is a protease.

Title: LUMINOGENIC AND NONLUMINOGENIC MULTIPLEX ASSAY

---

52. (Withdrawn) The method of claim 49 wherein the second enzyme does not react substantially with the first substrate.
53. (Withdrawn) The method of claim 49 wherein the first enzyme does not react substantially with the second substrate.
54. (Withdrawn) The method of claim 49 wherein the second substrate or the product of the reaction between second substrate and the second enzyme is fluorescent.
55. (Withdrawn) The method of claim 49 wherein fluorescence is employed to detect the presence or amount of the second enzyme or cofactor.
56. (Withdrawn) The method of claim 49 wherein at least one enzyme is a caspase.
57. (Withdrawn) The method of claim 49 wherein one of the substrates is a substrate for trypsin or tryptase.
58. (Withdrawn) The method of claim 49 wherein the sample is a cell lysate.
59. (Withdrawn) The method of claim 58 wherein the sample is a cellular sample that is treated with a cell death inducing agent prior to lysis.
60. (Withdrawn) The method of claim 49 wherein the sample comprises intact cells.
61. (Withdrawn) The method of claim 49 wherein the third enzyme is a luciferase.
62. (Withdrawn) The method of claim 61 wherein the luciferase is a beetle luciferase.



63. (Withdrawn) The method of claim 62 wherein the second substrate or the product of the reaction between second substrate and the second enzyme is fluorescent.
64. (Withdrawn) The method of claim 49 wherein the sample is contacted with the first substrate before the second substrate.
65. (Withdrawn) The method of claim 49 wherein the sample is contacted with the second substrate before the first substrate.
66. (Withdrawn) The method of claim 49 wherein the sample is simultaneously contacted with the first and the second substrates.
67. (Withdrawn) The method of claim 49 wherein the presence or amount of the first enzyme or first cofactor is detected before the presence or amount of the second enzyme or second cofactor is detected.
68. (Withdrawn) The method of claim 49 wherein the presence or amount of the second enzyme or second cofactor is detected before the presence or amount of the first enzyme or first cofactor is detected.
69. (Withdrawn) The method of claim 68 wherein the second substrate or the product of the reaction between second substrate and the second enzyme is fluorescent.
70. (Withdrawn) The method of claim 69 wherein the second substrate or the product of the reaction between the second substrate and the second enzyme comprises ethidium bromide, fluorescein, Cy3, BODIPY<sup>TM</sup>, a rhodol, Rox, 5-carboxyfluorescein, 6-carboxyfluorescein, an anthracene, 2-amino-4-methoxynaphthalene, a phenalenone, an acridone, fluorinated xanthene derivatives,  $\alpha$ -naphthol,  $\beta$ -naphthol, 1-hydroxypyrene,

Title: LUMINOGENIC AND NONLUMINOGENIC MULTIPLEX ASSAY

---

coumarin, 7-amino-4-methylcoumarin (AMC), 7-amino-4-trifluoromethylcoumarin (AFC), TEXAS RED™, tetramethylrhodamine, carboxyrhodamine, rhodamine, cresyl, rhodamine-110 or resorufin.

71. (Withdrawn) The method of claim 15, 16 or 49 wherein the cofactor is ATP.
72. (Withdrawn) The method of claim 49 wherein the presence or amount of the second enzyme is detected by contacting the sample with a fourth enzyme and third substrate for a reaction between the product of the reaction between the second substrate and second enzyme which yields a fluorogenic product.
73. (Withdrawn) A method to detect the presence or amount of a molecule for a first enzyme-mediated reaction, comprising:
  - a) contacting a sample which comprises cells which express a fluorescent protein with a reaction mixture for the first enzyme-mediated reaction, wherein a reaction mediated by the first enzyme yields a luminogenic product; and
  - b) detecting the presence or amount of the molecule and the presence or amount of the fluorescent protein in the sample.
74. (Withdrawn) The method of claim 73 wherein the molecule is a substrate.
75. (Withdrawn) The method of claim 73 wherein the molecule is a co-factor.
76. (Withdrawn) The method of claim 73 wherein the molecule is an enzyme.
- 77-84. (Canceled)
85. (Previously Presented) The method of claim 8 wherein the bioluminescence increases in

the presence of the first molecule.

86. (Previously Presented) The method of claim 1 wherein the bioluminogenic product is a substrate for a beetle luciferase.
87. (Currently Amended) The method of claim 1 wherein the fluorogenic product comprises fluorescein, Cy3, BODIPY™ (4,4-difluoro-1,3-dipropyl-4-bora-3a,4a-diaza-s-indacene), a rhodol, Rox, 5-carboxyfluorescein, 6-carboxyfluorescein, an anthracene, 2-amino-4-methoxynaphthalene, a phenalenone, an acridone, fluorinated xanthene derivatives,  $\alpha$ -naphthol,  $\beta$ -naphthol, 1-hydroxypyrene, coumarin, 7-amino-4-methylcoumarin (AMC), 7-amino-4-trifluoromethylcoumarin (AFC), TEXAS RED™ (sulforhodamine 101), tetramethylrhodamine, carboxyrhodamine, rhodamine, cresyl, rhodamine-110 or resorufin.
88. (Previously Presented) The method of claim 1 wherein the first molecule is a protease.
89. (Previously Presented) The method of claim 1 wherein the second molecule is a protease.
90. (Previously Presented) The method of claim 1 wherein one of the molecules is a glycosidase, phosphatase, kinase, dehydrogenase, peroxidase, sulfatase, peptidase, or hydrolase.
91. (Previously Presented) The method of claim 1 wherein the presence or amount of the first and second molecules is detected simultaneously.
92. (New) The method of claim 1 wherein the presence or amount of the first and second molecule is detected sequentially.